For research use only

ISO9001

HiPi Plus DNA Polymerase

Product Description	Quantity	Cat. No.	Remarks
HiPi Plus DNA Polymerase	500 unit	EBT-1007	5 unit/µl
	500 unit	EBT-1010	with 1 ml dNTP mix

Description

HiPi Plus DNA Polymerase is suitable for a high fidelity amplification of DNA fragments. This enzyme is designed for a reliable amplification of long (up to 10 kbp for lambda DNA) and complex targets with a robust yield and high specificity. HiPi Plus DNA Polymerase generates a mixture of PCR products with blunt end and 3′-dA overhangs.

Storage Buffer

5 unit/µl in 20 mM Tris-HCl, pH8.0, 100 mM KCl, 0.1 mM EDTA, 1 mM DTT, 0.5% Tween 20, and 50% Glycerol.

Application(s)

High fidelity PCR, RT-PCR, genomic PCR, long PCR.

Unit Definition

One unit is defined as the amount of enzyme required to catalyze the incorporation of 10 nmole of dNTP into an acid-insoluble form in 30 min at 72℃.The reaction conditions are : 25 mM TAPS, pH9.3, 50 mM KCl, 2 mM $MgCl_2$, 1 mM β-mercaptoethanol, 200 µM each dNTPs, 100 µM [α-^{32}P]dCTP, 12.5 µg activated calf thymus DNA in a total volume of 50 µl.

10x Reaction Buffer

500 mM Tris-HCl, pH9.0, 160 mM $(NH_4)_2SO_4$, 35 mM $MgCl_2$, 1% Tween 20, 1 mg/ml BSA.

QC tests

Activity, SDS-PAGE purity, performance tests.

Storage Condition

Store at -20℃.

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Standard Protocol

1. Prepare 50 µl PCR solution as follows:

PCR grade distilled water :	- µl
10x reaction buffer :	5 µl
10 mM dNTP mix (2.5 mM each) :	4 µl
Primer (10 pmol/µl) :	1 µl each
Template :	1-10 ng
HiPi Plus DNA Polymerase :	0.5 µl (2.5 unit)

Adjust final vol. to 50 µl with PCR grade distilled water

* Always, HiPi Plus DNA Polymerase should be added last to the mixture
* If you are amplifying a fragment larger than 2 kbp and with high GC content, add 5 µl of 10x Q buffer into the PCR mix. It will greatly improve reaction specificity. Q buffer is helpful for GC rich template and long template.

2. Set PCR cycling as follows :

Initial denature at 95°C : 3 min

		< 1 kbp	1-10 kpb
Denature	95°C	30 sec	30 sec
Anneal	Tm-4°C	30 sec	30 sec
Extend	72°C	45 sec	30-60 sec/kbp

* 25-45 PCR cycles
* You can also use two step cycle for > 5 kbp amplification (denaturation at 95°C and annealing/extension at 68°C)

Trouble-Shooting

1. No products
   - Confirm your template is intact : Try another reaction with a result assured primer pair and templates
   - Be sure all the component are correctly added and working well : Sometimes low graded dNTP may inhibit the reaction, and degraded primers can result in low sized PCR fragments
2. Smear bands or smeared background
   - Reduce template concentration : High concentration of template can lead to smearing of PCR products. Generally, 1-10 ng of plasmid DNA is working well
   - Increase annealing temperature
3. Non-specific bands
   - Increase annealing temperature
   - Consider using PCR additives, like 1-2% DMSO and 0.5-1x Q buffer
   - Confirm specificity of your primers
4. Low yield
   - Increase enzyme concentration in the reaction
   - Increase PCR cycle number
   - Be sure appropriate concentration of your template is added